STUDIES ON THE BIOLOGY OF PARACOPIDOSOMOPSIS II. SPERMATOGENESIS OF MALES REARED FROM UNFERTILIZED EGGS.[1]

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In the first paper of this series it was pointed out that the unfertilized egg of *Paracopidosomopsis floridanus* produces a polyembryonic brood of males. It is the purpose of this paper to show that the germ cells of such males are characterized by the haploid number of chromosomes. As in many other Hymenoptera, the presence of the half number is due to the fact that in the parthenogenetic development of the matured egg there is no compensatory process for restoring the full number. As a consequence of the reduced number of chromosomes in these males, the maturation divisions are modified in such a way that two, instead of four, spermatozoa are produced from each first spermatocyte.. In the somatic cells of these males the haploid number, subject to certain variations, also prevails; but this is a subject that will be considered later.

Material and Methods.

All of the material used in the study of spermatogenesis has been taken from animals reared under experimental conditions. It consists of two large broods of larvæ and a series of one hundred pupæ taken from a mummified carcass of the host. The two larval broods were preserved forty-eight and twenty-four hours, respectively, prior to the time at which pupation would have occurred. The series of pupa stages were preserved at close intervals, beginning twenty-four hours after the formation of the carcass.

The three broods yielding this material resulted from an experiment designed to determine whether the unfertilized egg

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 131.

developed into a brood of males. A summary of the experiment is as follows:

Host eggs laid the night of November 7.
Each egg parasitized by one oviposition of an unfertilized female, 2:30 P.M., Nov. 8.
Caterpillars hatched Nov. 13.
First brood of parasites preserved Dec. 4.
Second brood of parasites preserved Dec. 5.
Carcass of third caterpillar formed Dec. 6.
Series of pupa stages from carcass preserved Dec. 7–19.
Brood of 1842 male parasites emerged Dec. 25.

We have used a great variety of fixing fluids on polyembryonic material, but for the cytology of male germ cells no reagent has been found that equals Bouin's picro-formol-acetic mixture to which crystals of urea have been added. Preparations of the gonads fixed in this fluid and treated with Heidenhain's iron hæmatoxylin, counterstained with Orange G, give very fine results. However, this method of fixation has the disadvantage of not being adapted to the staining technique usually employed to bring out certain cytoplasmic structures, such as the mitochondria. Nevertheless, we have used it almost exclusively because of our especial interest in the chromosomes.

Even with the use of the best methods of technique, Hymenoptera material is never as favorable for the study of chromosomes as that of many other insects, notably the Hemiptera and Orthoptera. In certain respects, the germ cells of *Paracopidosomopsis* are more favorable for cytological study than those of many other species of the order, but even in this species the chromosomes are, at certain stages, greatly elongated, with a consequent tendency to become entangled with one another. This often makes difficult the determination of the exact number. The difficulty is somewhat increased by the smallness of the cell, which at the end of the growth period does not exceed a diameter of seven or eight microns. The best counts of chromosome numbers are obtained in somatic cells, because in these cells the metaphase plate is flat and the chromosomes are more scattered than in the spermatocytes. In any event, one does not experience difficulty in distinguishing a metaphase plate

with the haploid number of chromosomes from one having the diploid number.

The Spermatogonia.

We have not attempted to follow closely the course of development of the male gonads in the larva. At the earliest stage at which the larva can be recognized as a male, each testis consists of a solid spherical mass of cells. Histologically, there are two kinds of cellular elements, germ cells and epithelial cells. During the course of further development the gonad elongates, and at the same time cyst formation occurs. This process is completed by the end of the larval period.

At no time during the development of the gonad does one find any great number of spermatogonial divisions. Four or five mitotic figures are the most that will be found at any one time in a given testis.

The period of multiplication ends sometime between forty-eight and twenty-four hours prior to pupation. Of the two larval broods mentioned above, the one preserved forty-eight hours before the time of pupation has a few larvæ showing spermatogonial divisions, while the one fixed twenty-four hours later has no divisions.

Fig. 1 shows a metaphase plate of one of the dividing spermatogonia. The plate has eight elongated chromosomes, one of which shows the beginning of a longitudinal split. We have concluded from a study of all of our material of both males and females that eight represents the haploid number of chromosomes.

The Growth Period.

The growth period begins shortly after the cessation of the spermatogonial divisions, and extends through the fourth day after pupation. At the beginning of this period all of the germ cells in a given cyst are in the same stage of development. In section each cell appears wedge-shaped, with its slightly curved base lying against the outer membrane and its apex directed toward the center of the cyst. The apexes of all the cells in the cyst are usually connected by a common mass, which is apparently the remains of the interzonal fibers of the last spermatogonial division.

The growth stage is characterized by the presence of a conspicuous nucleolus, which occupies an excentric position in the nucleus (Figs. 2–4). At first the nucleolus is somewhat diffuse, but later its outline becomes sharp and distinct (Fig. 2). It frequently has several less deeply stained areas lying at its center (Fig. 3). We have good reasons for believing that the nucleolus is chromatin in character.

One of the most striking changes to occur in the cell during growth, is the appearance of certain cytoplasmic inclusions, which stain intensely black with iron hæmatoxylin. These are first seen about seventy-two hours after pupation, when they appear as dark, elongated areas in the cytoplasm. Later, as the cell enlarges, the areas become more definite and appear as seen in Fig. 3. These structures soon disappear (Figs. 4, 5), although some of them may persist until after the first maturation division is well advanced (Figs. 6, 8). We have not attempted to determine the origin and nature of these cytoplasmic inclusions; but, judging from the work of Meves on the bee, they are probably mitochondrial in character.

The presence of these inclusions greatly interferes with the study of the changes which now take place in the cell. Especially is this true with reference to those changes involving the behavior of the centrosomes. We shall therefore confine our account to the changes in shape of the cell which occur during the growth period. As already stated, all of the cells of a cyst are at first united at their pointed ends by the interzonal connections. In some cells these connections are soon lost, and each cell then becomes polyhedral in outline (Figs. 2, 3), and finally spherical. In other cells the interzonal connections apparently persist throughout the entire growth period, and after these are finally severed, the pointed end of each primary spermatocyte contains the remains of the original interzonal connection in the form of an "interzonal body" (Mark and Copeland).

At the end of the growth period many cells are met with which possess a delicate process. It is difficult to demonstrate the presence of a centrosome at the end of this process (Fig. 4). With reference to the position of the cell in the cyst, the process may lie on any side of the cell. It is not clear whether such a

process is formed in every cell. If it is of constant occurrence, the period of its existence must be very brief; for at the beginning of the first spermatocyte division, which soon follows, it is entirely absent.

A similar process has been described for the young spermatocytes of several different Hymenoptera, but the most careful and detailed work on it is that of Meves ('07) on the honey bee. According to Meves, the process in the bee is formed through the influence of one of the primary centrosomes ("Hauptcentriolen"), which arise by division from the original centrosome of the cell. The process with its centrosome is soon withdrawn into the cell. One of the several secondary centrosomes ("Nebencentriolen") which had previously arisen by division from the primary centrosomes, then participates in the formation of the mitotic figure responsible for the production of the so-called polar body.

While it is not possible in our material to demonstrate all of these fine points, yet it is highly probable that the cytoplasmic process in the germ cells of *Paracopidosomopsis* has a similar history to that described by Meves for the germ cells of the bee.

First Maturation Division.

Preparation for the first maturation division is made manifest by changes in the nucleus. It is also made evident by changes in the shape of the cell, especially in the spermatocytes which have early lost their interzonal connections. In cells in which these connections persist up until the end of the growth period, the so-called polar body, or rudimentary second spermatocyte, is budded off from the side of the cell that had recently had the connection. In the other spermatocytes a blunt protrusion arises at one side (Fig. 5), which soon develops into a pointed process (Fig. 6). At the pointed end thus formed there is present a tiny centrosome, from which radiate a number of delicate fibers toward the nucleus. At the opposite, or blunt end of the cell, a similar centrosome with its fibers is also present.

The most important change in the nucleus concerns the nucleolus, which during the first half of the growth period remains a deeply staining, spherical mass (Figs. 2, 3). A number

of spherical granules arise at its periphery, giving it the appearance of a lobulated structure (Fig. 4). Eventually the nucleolus breaks up into a number of these large granules, which lie in a finely granular matrix (Fig. 5). Apparently these larger granules coalesce to form the chromosomes (Fig. 7). From a careful study of this particular stage, one can not escape the conclusion that the chromosomes arise from the nucleolus.

A somewhat similar method of origin of chromosomes has been described by Meves and Duesberg ('08) in the male germ cells of *Vespa crabro*. They say: "Das Herannalien der ersten Reifungsteilung macht sich dadurch bemerkbar, das im Kern in der Umgebung des grösseren Nucleolus immer mehr Chromatinkörner auftreten. Der Nukleolus selbst wird dabei immer kleiner. Man gewinnt den Eindruck, dass seine Substanz in diejenige der Chromatinkörner übergeht." There is one important difference between the two forms. In *Paracopidosomopsis* the nucleolus gradually but completely disappears as the chromosomes are formed, while in *Vespa* a small body still remains after the chromosomes are organized.

During the organization of the chromosomes the nucleus elongates in the direction of the long axis of the cell. An imperfect intranuclear spindle then arises, and upon this the chromosomes tend to take up an equatorial position (Figs. 6, 8). They are usually so closely massed together that it is impossible to make an exact determination of their number. Fig. 6 shows a very interesting case, in which a single curved chromosome has moved to the upper end of the nucleus, where it lies in contact with the inner surface of the nuclear membrane. On account of the massed condition of the chromosomes, it is not possible to tell whether this particular chromosome is the product of a recent division, and has a sister chromosome lying within the mass. However, this is an isolated case, and it is clear that the chromosomes do not normally divide in the first spermatocyte division. Instead, only the cytoplasm undergoes division, and this in a very adequal manner (Figs. 7, 8).

The constriction of the cytoplasm begins about one-third the distance from the pointed end of the cell (Fig. 7) and finally results in cutting off a small, knob-like mass entirely free of

chromatin (Fig. 9). This abortive division is homologous to the first spermatocyte division which normally occurs in other forms, and the two unequal cells thus produced are to be regarded as equivalent to second spermatocytes.

The small non-nucleated cell, or mass of cytoplasm, soon degenerates, although in some cases it may persist until the second maturation division is well advanced. All stages in the disintegration of the mass are to be seen in a single cyst. The fragmenting masses lie in the interstices of the dividing second spermatocytes.

The most interesting point to be noted in connection with this abortive maturation division is the fact that nothing comparable to a synapsis of chromosomes occurs. The absence of this phenomenon, which is universal in the spermatogenesis of males arising from fertilized eggs, is undoubtedly due to the fact that the unmatured, male germ cell of *Paracopidosomopsis* possesses but the haploid number of chromosomes. The function of the first maturation division is to bring about a reduction from the diploid to the haploid number of chromosomes; but the males of this species, by virtue of their origin from matured, but unfertilized eggs, already possess the half number, and consequently, the first maturation results in a feeble or abortive attempt at a division.

Second Maturation Division.

Throughout the entire period occupied by the first maturation, the nuclear membrane remains intact (Figs. 5–9). At the end of this period the incomplete intranuclear spindle disappears, as do also the fibers which radiate from the centrosome included in the small cytoplasmic mass (Fig. 9). On the contrary, the aster, which lies at the lower side of the nucleus in the true second spermatocyte, persists. It is highly probable that this aster, by a division of its centrosome, gives rise to the second maturation spindle, as is the case in the European hornet.

In the brief interim between the first and second maturations, the nucleus enters a rest stage. The chromatin recedes to that side of the nucleus lying farthest from the cytoplasmic bud, and becomes so massed that it is impossible longer to distinguish

clearly individual chromosomes (Fig. 9). In the stages immediately following this one, every phase in the reorganization of the chromosomes is clearly demonstrable. In having a definite resting stage between the abortive and the true division, *Paracopidosomopsis* resembles *Neuroterus* (Doncaster, '09), but differs from such forms as the bee and the wasp.

After the cytoplasmic bud is cut off, the second spermatocyte becomes spherical in outline, and a conspicuous maturation spindle is formed (Fig. 12). The reorganized chromosomes are drawn into the equatorial position on this spindle. Clear polar views are difficult to find, owing to the fact that the chromosomes are elongated and frequently twisted (Fig. 12). In the clearest cases observed, the number of chromosomes is seen to be eight (Figs. 10, 11).

The axis of the second maturation spindle bears no definite relation to that of the first maturation spindle. This was determined by studying those second spermatocytes to which the cytoplasmic bud remains for some time slightly attached. It was found that the axis of the second spindle has no definite relation to the point of attachment.

In the second division the chromosomes split lengthwise, and the daughter chromosomes pass to the opposite poles of the spindle (Fig. 14). It is frequently easy to count the eight daughter chromosomes passing to the one or the other of the poles, but cells in which both daughter groups can be counted are not often met with (Fig. 15). We conclude from these observations that all of the chromosomes divide in the second maturation. While in the anaphases the chromosomes are often well scattered, yet we have found no evidence of a distinctly "advancing" or "lagging" member, such as might indicate that a particular chromosome had failed to divide. From this it follows that the two spermatids resulting from the second division will be alike. That is to say, the spermatozoa in *Paracopidosomopsis* will not be dimorphic.

In passing to the poles of the spindle the chromosomes keep their long axes parallel to the long axis of the spindle (Figs. 15, 16), and upon reaching the pole fuse to form a solid, deeply staining mass of chromatin out of which the spermatid nucleus arises (Figs. 17, 18).

After the division of the cytoplasm, the two spermatids remain connected for some time by means of the interzonal fibers (Fig. 18). After they separate, each differentiates into a typical spermatozoön (Figs. 19, 20). We shall not discuss further the subject of the metamorphosis of the spermatids, except to emphasize the point that both spermatids must form functional germ cells, as we have found no evidence of degenerating spermatids or spermatozoa.

Comparison with other Hymenoptera.

Studies on the spermatogenesis of Hymenoptera have shown that there is a striking modification of the maturation process in those species in which sex-determination is supposed to be in accordance with the Dzierzon theory. Instead of four spermatozoa arising from each first spermatocyte, only one or two matured germ cells develop. In one group of these Hymenoptera both spermatocyte divisions are abortive; the first division cutting off a small cytoplasmic bud free of chromatin, and the second division a small bud which receives half the chromatin. There is thus produced but a single spermatid, and consequently only one spermatozoön. In the second group the first division is likewise abortive, producing a cytoplasmic bud, but the second division is equal, producing two similar spermatids, which metamorphose into spermatozoa.

To the first of these groups belongs the honey bee, as has been shown by the work of Meves ('03, '07), Mark and Copeland ('06), and Doncaster ('06, '07). To this class there also probably belongs the solitary bee (*Osmia cornuta*) worked on by Armbruster ('13).

The second group includes a number of different species, of which the following may be mentioned: *Xylocopa violacea* (Granata, '10, '13). *Neuroterus lenticularis* (Doncaster, '09), *Vespa crabro* (Meves and Duesberg, '08), *Vespa maculata* (Mark and Copeland, '07), *Vespa germanica* (Meves, '03), *Camponotus herculaneus* (Meves and Duesberg, '08, and Lams, '08), *Dryophanta erinacei* (Wieman, '15). *Paracopidosomopsis floridanus* also clearly belongs to this class.

While the various investigators report differences as to details

in the two types of spermatogenesis outlined above, yet there is great uniformity in their interpretations of the general character of the processes involved. Our own work on *Paracopidosomopsis* indicates that the spermatogenesis of this species is very similar, not only as regards the general character of the process, but also as regards many details, to the maturations of the European hornet, *Vespa crabro*, as shown by the work of Meves and Duesberg.

AUSTIN, TEXAS,
February 6, 1917.

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DESCRIPTION OF FIGURES.

All of the figures are camera drawings made at table level, with the Zeiss 2 mm. lens and No. 12 compensating ocular. The figures thus outlined were enlarged three times by the aid of an enlarging camera, and then reproduced with one third off. They are therefore magnified about 3857.

PLATE I.

FIG. 1. Metaphase plate of last spermatogonial division.

FIG. 2. Stage at the beginning of growth period.

FIG. 3. Stage at the mid-growth period, showing the conspicuous cytoplasmic inclusions.

FIG. 4. Spermatocyte with delicate cytoplasmic process.

FIG. 5. Elongation of the cell in preparation for the first spermatocyte division.

FIG. 6. Stage showing the imperfect intranuclear spindle.

FIG. 7. Stage showing the beginning of the constriction which results in cutting off the cytoplasmic bud.

FIG. 8. A later stage showing the same process.

FIG. 9. Stage showing the end of the first maturation division. The cytoplasmic bud has just been cut off.

FIG. 10. Metaphase plate of the second spermatocyte.

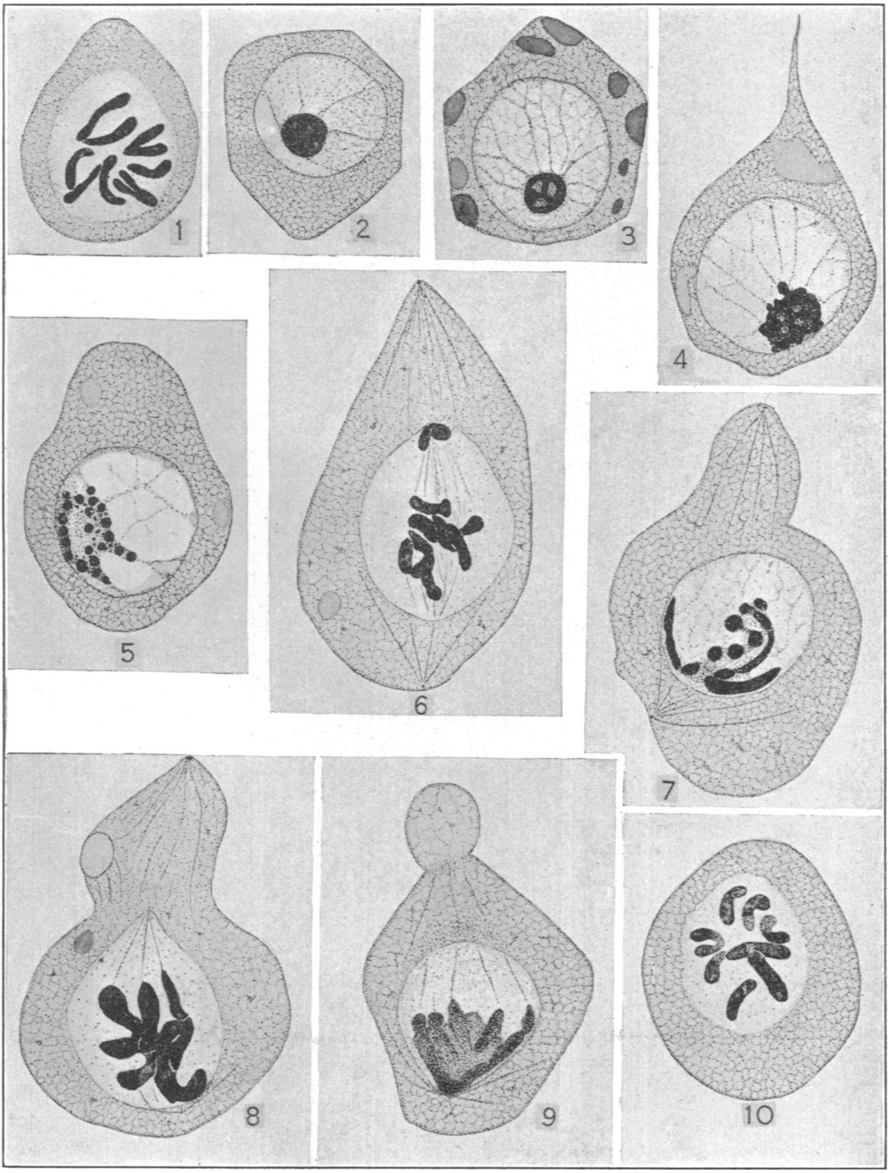

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PLATE II.

FIG. 11. Metaphase plate of the second spermatocyte.

FIG. 12. Side view of the second maturation spindle, showing four twisted chromosomes.

FIG. 13. A section through one side of the spindle, showing two chromosomes that have just divided. Parts of two other chromosomes are also present.

FIG. 14. A similar section, showing the complete division of four chromosomes.

FIG. 15. Anaphase stage, showing eight daughter chromosomes passing to each pole.

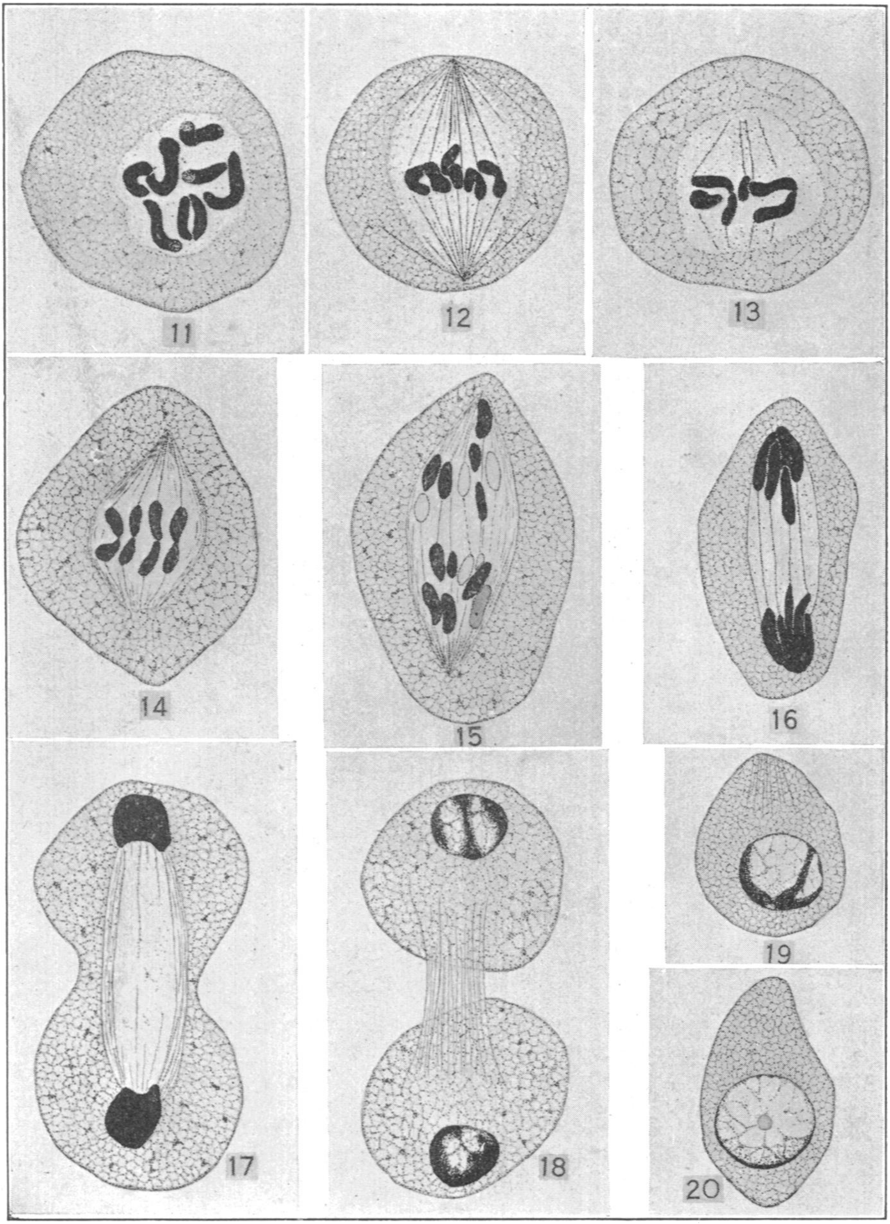
FIG. 16. Late anaphase stage.

FIG. 17. Telophase stage.

FIG. 18. The two spermatids, still connected by means of the interzonal fibres.

FIG. 19. A completely separated spermatid.

FIG. 20. A later stage of the spermatid.



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